Potent low toxicity inhibition of human melanogenesis by novel indole-containing octapeptides.

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Authors: A A Ubeid, S Do, C Nye, B M Hantash

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Public Summary:
An abnormal production and accumulation of melanin is characteristic of a large number of skin disorders, including postinflammatory hyperpigmentation and melasma. Melanin is a photoprotective biopolymer synthesized by melanocytes in discrete organelles known as melanosomes. Once filled with melanin, melanosomes are transferred to keratinocytes, where they form a supranuclear cap to protect DNA against ultraviolet irradiation. Melanin forms through a series of oxidative reactions involving the amino acid tyrosine and the enzyme tyrosinase, a melanocyte-specific, multi-functional, glycosylated, copper-containing oxidase. The first two steps in the melanogenic pathway, the hydroxylation of L-tyrosine to 3-4- dihydroxyphenylalanine (L-dopa) and the oxidation of L-dopa to o-dopaquinone are catalyzed by the enzyme tyrosinase. It is believed that racial differences in skin color are primarily due to differences in tyrosinase activity. Melanocytes derived from African skin demonstrate up to ten times more enzymatic activity and melanin production than melanocytes from Caucasian skin . However, this is not due to a greater abundance of tyrosinase, as both skin types have been shown to contain a similar density of tyrosinase molecules. Based on these findings, targeting the enzyme tyrosinase is the most efficient and focused approach to treat pigmentary disorders. The most common pigmentary disorders for which patients seek treatment are melasma and post-inflammatory hyperpigmentation. Pharmacological agents that can reduce hyperpigmentation are of great interest clinically, and include hydroquinone (HQ), arbutin, kojic acid, vitamin C, retinol, azelaic acid, and numerous botanical compounds. Although HQ has been the mainstay treatment for hyperpigmentation, its cl inical potential has been complicated by a number of adverse reactions including contact dermatitis, irritation, transient erythema, burning, prickling sensation, leukoderma, chestnut spots on the nails, hypochromia and ochronosis. Moreover, HQ is potentially mutagenic to mammalian cells. HQ and its derivative arbutin are both catabolized to benzene metabolites with the potential for bone marrow toxicity. Together, these risks have led the European Committee (24th Dir. 2000/6/ EC) to ban the use of HQ in cosmetics. Therefore, the search for a safe and efficacious hypopigmenting agent is warranted. In the manuscript we discuss the development and validation of novel oligopeptides with potent inhibitory activity aga inst mushroom and human tyrosinase with minimal toxicity towards the major skin cellular constituents, melanocytes, keratinocytes, and fibroblasts. A library of short sequence oligopeptides was docked against the crystal structure of mushroom tyrosinase to screen for favorable binding free energies and direct interaction with the catalytic pocket. The inhibitory activity of the octapeptides and hydroquinone (HQ) was assessed using mushroom and human tyrosinase and melanin content via human primary melanocytes. Effects on cell viability and proliferation were determined using the MTI assay and cytotoxicity via trypan blue exclusion. Octapeptides P16-18 outperformed HQ, the benchmark of hypopigmenting agents, in all tested categories, and were shown to be potent competitive tyrosinase inhibitors with minimal toxicity towards the major cell types of human skin.The findings in our study suggest that all three novel octapeptides may serve as safe and efficacious replacements of HQ for the treatment of pigmentary disorders.

Scientific Abstract:
BACKGROUND: Abnormal production and accumulation of melanin are characteristics of a number of skin disorders, including postinflammatory hyperpigmentation and melasma. Our objective was to develop and validate novel oligopeptides with potent inhibitory activity against mushroom and human tyrosinase with minimal toxicity toward melanocytes, keratinocytes, and fibroblasts. METHODS: A library of short sequence oligopeptides was docked against the crystal structure of mushroom tyrosinase to screen for favorable binding free energies and direct interaction with the catalytic pocket. The inhibitory activity of the octapeptides and hydroquinone (HQ) was assessed using mushroom and human tyrosinase and melanin content via human primary melanocytes. Effects on cell viability and proliferation were determined using the MTT assay and cytotoxicity via trypan blue exclusion. RESULTS: Octapeptides P16-18 outperformed HQ, the benchmark of hypopigmenting agents, in all tested categories. Prolonged incubation of human keratinocytes, fibroblasts, or melanocytes with 30-3000muM HQ led to 8- to 65-fold greater cell death than with octapeptides.

After 6d of incubation with 30muM HQ, we observed 70+/-3% and 60+/-2% cell death in melanocytes and fibroblasts, respectively, versus minimal toxicity up to an octapeptide concentration of 3mM. CONCLUSION: Octapeptides P16-18 are potent competitive tyrosinase inhibitors with minimal toxicity toward the major cell types of human skin. GENERAL SIGNIFICANCE: The findings in our study suggest that all three novel octapeptides may serve as safe and efficacious replacements of HQ for the treatment of pigmentary disorders.
